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(54) Pharmaceutical preparations for the treatment of sarcocystosis

(57) Methods for the preparation application by intravenous injection, and the storage and characteristics of pharmaceutical preparations for treating both the acute and chronic phases of Sarcocystosis, a disease that is caused by the presence of cysts of the Sarcocystis protozoa and like protozoa in an Intermediate Host (IH), is described.

The pharmaceutical preparation for the treatment of Sarcocystosis and like demyelinisation diseases, including BSE and CJD, in Intermediate Hosts caused by the Sarcocystis protozoa and like protozoa and their toxins, comprises as an active ingredient sodium or potassium iodide stabilized against the formation of hydriodic acid in an aqueous solution having a pH in the range 8.0 to 9.0 and in a form suitable for administration to a human or animal by injection, which preparation includes iodide ions, sodium ions and potassium ions in mole/mole ratios ranging from 0.01 to 0.05/18.07 to 26.40/0.015 to 0.032, respectively.

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PHARMACEUTICAL PREPARATIONS FOR THE TREATMENT OF SARCOCYSTOSIS

This invention relates to methods for the preparation for the application by intravenous injection, as well as the storage and characteristics, of pharmaceutical preparations for treating both the acute and chronic phases of Sarcocystosis, a disease that is caused by the presence of cysts of the Sarcocystis protozoa and like protozoa in an Intermediate Host (IH). Other similar demyelinisation diseases can be similarly treated including bovine spongiform encephalopathy (BSE) and its human equivalent Creutzfeldt-Jakob disease (CJD).

In particular, this invention relates to the preparation of pharmaceutical preparations for treating Sarcocystosis, a disease detrimental to the neuromuscular, productive and reproductive systems of farm animals. More particularly, pharmaceutical preparations will be disclosed which are produced by the methods described hereinafter, which can be stored ready for use in glass containers with two compartments, one with a solid active ingredient therein and the other compartment with a liquid vehicle for dilution of the solid active ingredient. In a preferred embodiment this separation of the preparation components during storage helps to prevent hydriodic acid formation, and thus guarantees the safety of the preparation at the time of use. Preferably the liquid vehicle contains substances that induce optimal penetration of the pharmaceutical preparation into the cysts caused by the disease and thereby help patients overcome the toxemia caused by the death of many parasites.

To date, the chemotherapy for the control of Sarcocystosis is of the nature of preventive treatment, such as the use

of salts of "amprolium" (the hydrochloride or chloride of 1-(4 amino-2 propyl) methyl picoline - a known anticoccidal agent) which are administered continuously by being mixed with the patients' drinking water. Its main purpose is to kill the parasites before they reach the patients' blood supply. In the chronic phase of the disease, the use of sulphur compounds such as "trimetoprin sulphur" (2,4-diamino-5-(3,4,5-trimethoxy benzyl) pyrimidine - a known antibacterial agent) for 8 to 10 weeks has been reported. By contrast, the present invention is useful for both of the two phases of the disease, and it entails the advantage that treatment requires only 4 days for eradication of the Sarcocystis protozoa and like protozoa from the patient.

In accordance with the present invention there is provided, a pharmaceutical preparation for the treatment of Sarcocystosis and like demyelinisation diseases, including BSE and CJD, in Intermediate Hosts caused by the Sarcocystis protozoa and like protozoa and their toxins, comprising as an active ingredient sodium or potassium iodide stabilized against the formation of hydriodic acid in an aqueous solution having a pH in the range 8.0 to 9.0, preferably 8.0 to 8.5 and in a form suitable for administration to a human or animal by injection, which preparation includes iodide ions, sodium ions and potassium ions in mole/mole ratios ranging from 0.01 to 0.05/18.07 to 26.40/0.015 to 0.032, respectively.

In the formulation and dosage of the present pharmaceutical preparations, the iodide concentration that is obtained in the inside of the parasite cysts is highly effective in attaining the desired parasiticidal effect. Using a preferred pharmaceutical preparation of the present invention, after treatment it has been found by biopsy that the number of encyst parasites in the muscle of a patient

decrease by at least 83%. In other words, a minimum of 83 out of 100 encyst parasites were found to have died because of the treatment. This advantage is based on the results of our research, which show that there is an optimum iodide ion concentration with respect to the concentration of the sodium and potassium ions present, which permits the preparation's penetration through the wall of both the cysts and the parasite, whereby the enzymes, which the Sarcocystis protozoa and like protozoa use for their metabolism, are denatured.

Preferably magnesium ions and chlorine ions are additionally present, optionally with calcium ions, and when present their mole/mole ratios should be in the ranges 1.2 to 2.0/18.0 to 26.8/0 to 0.7, respectively. Desirably the range for magnesium is 1.25 to 1.60. With this preferred composition the pharmaceutical preparations of the present invention can eliminate the toxin effects, by concentration competition generated at the cellular pump points for the sodium/potassium, calcium/ sodium and other related ion pumps. The most preferred ratio of ions mole/mole is: iodide 0.03/Sodium 19.02/Potassium 0.03/Calcium 0.53/Magnesium 1.34/Chlorine 19.19 in an alkaline medium (pH 8.25).

The pharmaceutical preparations of the present invention assume greater importance if it is taken into account that humans behave as Final Hosts (FH) for the Sarcocystis protozoa and like protozoa.

To date, the evidence of the infection in man with the parasite known as Sarcocystis has been usually found accidentally through histopathological examinations. Protozoa of the Sarcocystis genus behaves like an enzoonosis, and has been reported all over the world as a

causal agent of many pathologies in man. This parasite mainly affects lower strata people with deficient nutrition although any person may be susceptible to it.

Protozoa of the Sarcocystis genus were reported for the first time in 1843 when a researcher by the name of Miescher found tubules in the skeletal muscle of a house mouse. Doctors Rommel and Heidorn in 1972 found that the protozoa's life cycle was heterogeneous, with the asexual phase in the prey, (the IH) and the sexual phase in the pillager, (the FH). At present, 122 species of the protozoa have been identified, 56 of which are known to have two hosts. From the zoonosis point of view, those developing the asexual phase in humans are interesting and their FH are unknown to date. There are two species which develop the sexual phase in humans which are known.

A review of worldwide scientific literature does not report a parasite of the Sarcocystis genus being related in any way to multiple sclerosis, lateral amyotrophic sclerosis and the syndrome of chronic fatigue. Instead, a literature review shows that multiple sclerosis may be considered to be related to alterations in the immune system, mainly with alterations of the Tcd4 lymphocytes and rupture of the haematoencephalic barrier.

However, we have found that infection due to Sarcocystis also gives rise to symptoms such as muscle spasms, intermittent diarrhoea and chronic fatigue, even to multiple sclerosis. These symptoms are characteristic of demyelinisation diseases, and thus we conclude that there is a link with Sarcocystosis. In our research it has been determined that the pathogeny of this organism is caused by a toxin from a parasite of a Sarcocystis-like protozoa. The isolation of the toxin is described in U.K. Patent

Application No. 9603304.8. Further research has established that because of the underlying similarities between Sarcocystosis and the other diseases and symptoms described above, the mechanism by which the pharmaceutical preparation of the present invention operates is equally effective against these other diseases and symptoms. Indeed, treatment with the pharmaceutical preparation of the present invention has been found to cause re-myelinisation to take place. There has been much speculation recently about the causative agent in BSE and its human equivalent CJD, and interest has been focused on a prion protein found in the brain tissues of victims of these diseases. However, the close similarity of characteristics between the postulated prion and the toxin produced by Sarcocystis protozoa and like protozoa leads us to conclude that these causative agents are in fact the same, and therefore a pharmaceutical preparation, such as that of the present invention, which is effective against Sarcocystosis and like diseases will also be effective against BSE and CJD.

The present invention provides pharmaceutical preparations which are easy to use and which can control the parasites existing in the cysts of the Sarcocystis protozoa and like protozoa to be found in the IH, by using an aqueous iodide solution stabilised in an alkaline solution having a pH of from 8.0 to 9.0 and with concentrations of cations and anions in the solution that will inactivate the effect of the toxin of the Sarcocystis protozoa and like protozoa.

Any source of alkali is acceptable provided that it is suitable for an injectable pharmaceutical preparation, such as an alkali metal or alkaline earth metal carbonate, bicarbonate or lactate.

The preferred pharmaceutical preparations of the present invention consist of the following ingredients:-

A. **Natrium chloratum (sodium chloride NaCl)**

B. **Natrium lacticum (sodium lactate CH_3-CHOH-COONa)**

C. Kalium chloratum (potassium chloride KCl)

D. Calcium chloratum crystallisatum [$6H_2O$] (hydrated calcium chloride $CaCl_2.6H_2O$)

E. Magnesium chloratum [$6H_2O$] (hydrated magnesium chloride $MgCl_2.6H_2O$)

F. Potassium iodide (KI)

G. Natrium bicarbonicum (sodium bicarbonate $NaHCO_3$)

H. Aqua dist., csp (triple distilled water)

In order to help prevent the formation of hydriodic acid the potassium iodide is preferably not added until just prior to administration.

Preferably these ingredients are mixed together as follows, all of the processes being conducted at room temperature.

Liquid vehicle preparation:

Measure the required volume of triple distilled water to prepare the final volume of vehicle which is required.

Weigh the different solid components in the following ratios by weight per 100 ml of the liquid vehicle:

Natrium chloratum	**(NaCl)**	**950 mg**
Natrium lacticum	**(CH_3-CHOH-COONa)**	**305 mg**
Kalium chloratum	**(KCl)**	**80 mg**
Calcium chloratum crystallisatum [$6H_2O$]	**($CaCl_2.6H_2O$)**	**40 mg**
Magnesium chloratum [$6H_2O$]	**($MgCl_2.6H_2O$)**	**80 mg**

Mix all the components and dissolve in triple distilled water and sterilize the solution by filtration through a 0.2 micron membrane.

This solution is then stored in glass vials in accordance with the volume to be used and should be maintained at room temperature.

The active ingredient is prepared as follows:

Weigh the potassium iodide and the sodium bicarbonate in the proportion of 50:1 weight/weight respectively, mix and store in amber glass vials in the amounts required. The final pH of the pharmaceutical preparation when ready for administration must be between 8.0 and 9.5, preferably about 8.25.

When considering storage, it must be borne in mind that the preferred ratio of active ingredient/vehicle is 51 grams to 1,000 millilitres, respectively. This type of storage, in two glass vials, at least one of which should be amber, is preferred in order to achieve the desirable long term stability for the iodide component and in order to maximise the pharmaceutical effect of the preparations of the invention.

Administration procedure:

In order to prepare the described pharmaceutical preparation for administration, the active ingredient is diluted with the liquid vehicle in the proportion described above. Between 4 and 7 millilitres of the diluted preparation is administered by intravenous injection for each 100 kilograms of live weight of animal per day over a period of 4 continuous days.

When reconstituted as described above, the pharmaceutical preparation is stable for about 20 days if stored in a cool, dry and dark place.

Since the pharmaceutical preparation of the present invention is effective against the other described similar diseases such as BSE because of its similar mode of operation, the same dilution and treatment regime is equally applicable for those other diseases.

An eradication programme for bovine Sarcocystosis in endemic regions will now be described.

After identifying the farm or region to which the programme is to be applied, all the IHs must receive treatment. Moreover, it is indispensable to set up controls over the excreta of all FHs, including humans, dogs and cats, which must be vaccinated against the Sarcocystis-like protozoa.

Subsequently, and depending on the degree of infection of the region, a programme of treatment should be carried out semi-annually or annually, until discharge of the oocysts in the pasture ground becomes imperceptible or nil. To accomplish this, it is indispensable that no FH eats raw or badly cooked meat. One possible option is to ensure that all raw meat is stored by freezing for at least 3 days.

To perform the above control and eradication programme in regions of milk production or cattle reproduction at reasonable cost, it is advisable to apply the treatment to all male calves in the region semi-annually or annually. Females in production should be treated from 20 to 30 days before birth.

In case of beef livestock it is preferable to apply a first treatment at the beginning of the fattening period and the next treatment one or two months before slaughter.

Results so far obtained for the treatment of IHs by administering the above described preferred pharmaceutical preparation of the present invention as described above:

1. Cows

The preparation has been administered to 2,600 bovine animals who either showed symptoms of Sarcocystosis or were known to be carriers of the disease, and the following results have been obtained:

The pregnancy rate has increased by 31.5%, resulting in 80.8% of females having more than 4 inseminations becoming pregnant with only one insemination after treatment.

Abortion cases have been reduced by 83.0%.

Heart afflictions were reduced by 93.6%, which fact was demonstrated by normalization of ASAT, ALAT, CPK and GAMMA GT.

Haemodynamic abnormalities were corrected in treated animals in a time span of 10 to 15 days. This correction was evidenced by normalization of the haematic parameters,

sodium, potassium and chloride ion concentrations and blood pH.

Diarrhoea was corrected in 97.3% of adults and 94.1% of calves.

Fattening was increased by an average of 28.0%.

Open days were reduced from 120 to 70 days.

Intervals between births decreased from 530 to 351 days.

Milk production was increased by 10 litres per day per animal.

Adverse results:

Only two dead cows were reported. Their deaths were correlated with septic processes arising from the presence of clostridium bacteria at the point where the animals were injected.

2. Horses

The preparation was administered to 110 equine animals. Unlike bovine animals, only horses showing symptoms of Sarcocystosis were treated. The treatment produced a recovery rate of 82.7% for these animals which regained all their physical functions.

CLAIMS:

1. A pharmaceutical preparation for the treatment of Sarcocystosis and like demyelinisation diseases, including BSE and CJD, in Intermediate Hosts caused by the Sarcocystis protozoa and like protozoa and their toxins, comprising as an active ingredient sodium or potassium iodide stabilized against the formation of hydriodic acid in an aqueous solution having a pH in the range 8.0 to 9.0 and in a form suitable for administration to a human or animal by injection, which preparation includes iodide ions, sodium ions and potassium ions in mole/mole ratios ranging from 0.01 to 0.05/18.07 to 26.40/0.015 to 0.032, respectively.

2. A pharmaceutical preparation as claimed in claim 1, wherein the iodide ion ratio ranges from 0.02 to 0.38.

3. A pharmaceutical preparation as claimed in claim 1 or claim 2, wherein the preparation includes magnesium ions and chlorine ions in mole/mole ratios ranging from 1.2 to 2.0/18.0 to 26.8, respectively.

4. A pharmaceutical preparation as claimed in claim 3, wherein the magnesium ion range is 1.25 to 1.60.

5. A pharmaceutical preparation as claimed in claim 3 or claim 4, wherein the preparation additionally contains calcium ions in a mole/mole ratio with the other ions ranging from 0 to 0.78.

6. A pharmaceutical preparation as claimed in claim 5 having a composition in which the mole/mole ratio of ions is: 0.03 iodide/ 19.02 sodium/ 0.03 potassium/ 0.53 calcium/ 1.34 magnesium/ 19.19 chlorine.

7. A pharmaceutical preparation as claimed in any one of the preceding claims having a pH of about 8.25.

8. A pharmaceutical preparation as claimed in any one of the preceding claims consisting essentially of the following ingredients:-

A. Natrium chloratum (sodium chloride NaCl)

B. Natrium lacticum (sodium lactate CH_3-CHOH-COONa)

C. Kalium chloratum (potassium chloride KCl)

D. Calcium chloratum crystallisatum [$6H_2O$] (hydrated calcium chloride $CaCl_2.6H_2O$)

E. Magnesium chloratum [$6H_2O$] (hydrated magnesium chloride $MgCl_2.6H_2O$)

F. Potassium iodide (KI)

G. Natrium bicarbonicum (sodium bicarbonate $NaHCO_3$)

H. Aqua dist., csp (triple distilled water)

9. A pharmaceutical preparation as claimed in claim 8 prepared ready for administration by dissolving in a liquid vehicle the active ingredient, wherein the liquid vehicle comprises a solution in triple distilled water of the following solid components in the following ratios by weight per 100 ml of the liquid vehicle:-

Natrium chloratum	(NaCl)	950 mg
Natrium lacticum	(CH_3-CHOH-COONa)	305 mg

Kalium chloratum	**(KCl)**	**80 mg**
Calcium chloratum crystallisatum [$6H_2O$]	**($CaCl_2.6H_2O$)**	**40 mg**
Magnesium chloratum [$6H_2O$]	**($MgCl_2.6H_2O$)**	**80 mg**

and wherein the active ingredient is a mixture of potassium iodide and sodium bicarbonate in a weight ratio of 50:1.

10. A pharmaceutical preparation as claimed in any one of the preceding claims, wherein the active ingredient and the aqueous solution into which it is dissolved are stored in separate containers and adapted for mixing together in the desired proportions when ready for administration, the administration rate being between 4 and 7 millilitres of the preparation per 100 kilograms of live weight of animal per day.

11. A pharmaceutical preparation for the treatment of Sarcocystosis and like demyelinisation diseases as claimed in claim 1 substantially as hereinbefore described.

The Patent Office

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Application No: GB 9615710.2 **Examiner:** Dr Carol Davies
Claims searched: 1-11 **Date of search:** 28 October 1996

Patents Act 1977
Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

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Int Cl (Ed.6): A61K 33/18

Other: ONLINE: WPI; CAS-ONLINE; DIALINDEX:Medicine, Vetsci

Documents considered to be relevant:

Category	Identity of document and relevant passage		Relevant to claims
A	GB 1076670	(CHUGAI SEIYAKU K K) see for example, Examples 2 and 5	

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.